

## Article

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**Non-destructive assessment of the oxidative stability of intact macadamia nuts during the drying process by near-infrared spectroscopy**

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22

## 23 Abstract

24 We have developed a rapid non-destructive method to assess the oxidative stability of intact  
25 macadamia nuts using near-infrared spectroscopy (NIRS). Intact macadamia nuts of the cultivars  
26 HAES 344 ‘Kau’, HAES 660 ‘Keaau’, IAC 4-12 B, and IAC Campinas B were harvested and  
27 immediately oven-dried for 4 days at 30 °C, 2 days at 40 °C, and 1 day at 60 °C to achieve 1.5%  
28 kernel moisture content. At each drying step nuts were withdrawn and their moisture content,  
29 peroxide value (PV), and acidity index (AI) determined. The best partial least square model for PV  
30 prediction was obtained using the Savitzky-Golay (SG) second derivative resulting in a standard  
31 error of prediction (SEP) of 0.55 meq·kg<sup>-1</sup> and a coefficient of determination ( $R^2_C$ ) of 0.57. The best  
32 AI prediction-model result was obtained using the SG second derivative (SEP = 0.14%,  $R^2_C$  = 0.29).  
33 Based on the maximum quality limits of 3 meq·kg<sup>-1</sup> for PV and 0.5% for AI, the SEP values  
34 represented 18% and 28%, respectively. Therefore, the prediction method can be considered useful  
35 since the errors are lower than the quality limits. Thus, NIRS can be used to assess the oxidative  
36 stability of intact macadamia kernels.

37

38 **Keywords:** peroxide value, acidity index, *Macadamia integrifolia* Maiden & Betcher, principal  
39 component analysis.

40

## 41 1. Introduction

42

43 The delicious mild flavor and crispy texture of lightly roasted macadamia kernels have made  
44 them one of the most appreciated nuts in the world (Wall, 2013). High quality kernels contain 72–  
45 78% oil (Cavaletto, 1983), of which more than 77% corresponds to monounsaturated fatty acids,

46 predominantly monounsaturated fatty acids, while only around 16% are saturated fatty acids (Wood  
47 and Garg, 2010). This composition plays an important role in their quality, particularly in the  
48 organoleptic properties that make macadamia nuts so desirable (Silva, Maximo, Marsaioli Jr. &  
49 Silva, 2007).

50 Due to their high oil content, macadamia nuts are very susceptible to the occurrence of  
51 rancidification in the kernels, which causes objectionable flavors and odors in food products  
52 (Ramalho & Jorge, 2006; Silva et al., 2011). In addition, macadamia nuts contain the low levels of  
53 tocopherol antioxidants ( $\sim 0.6\text{--}2.8\ \mu\text{g}\cdot\text{g}^{-1}$ ), (Wall, 2013).

54 Moisture content strongly influences early rancidity in macadamia nuts, and therefore, to  
55 retard rancidification and extend shelf-life, shelled nuts should be protected from moisture and  
56 oxygen during storage (Wall, 2013). Since the oxidative stability of the kernels is related to their  
57 moisture content, this parameter must be monitored to ensure drying to below 1.5% (Cavaletto,  
58 1981; Silva et al., 2005; Borompichaichartkul et al., 2009). At this moisture level the water activity  
59 ( $a_w$ ) is equal or less than 0.3, which represents the optimum point for oxidative stability of  
60 dehydrated foods (Cavaletto, 1981; Wall, 2013). In addition to this, the maximum stability of  
61 macadamia nut corresponds to the minimum integral entropy zone (Dominguez et al., 2007), which  
62 corresponds to a range of  $a_w$  from 0.36 to 0.44 (1.2-1.6% moisture). Therefore, 1.5% moisture  
63 content is fundamental to assure the oxidative stability of macadamia nuts.

64 Since freshly harvested nuts have a moisture content higher than 30% (Pankaew et al., 2016),  
65 drying should be started soon after harvesting to reduce the moisture content to prevent hydrolytic  
66 rancidity due to the nuts' high oil content (Mason and Wills, 2000) and/or mold development, such  
67 as *Colletotrichum gloeosporioides* and *Botrytis* sp. (Dierberger and Marino Netto, 1985).

68 The most useful analytical parameters to determine the degree of oxidation are peroxide value  
69 (PV) and acidity index (AI) (Wolff, 1997). PV is related to the formation of peroxides in unsaturated  
70 fats, due to the breaking of the double bonds, which generates short-chain volatile products

71 responsible for rancid odors. AI is related to the acidification of fats due to enzymatic reactions,  
72 generating free fatty acids that could have unpleasant taste (Wolff, 1997). PV and AI can change at  
73 high temperatures and can also depend on the cultivars. For example, PV changes on heating as it  
74 decomposes into highly unstable secondary oxidation products while AI is influenced by heating due  
75 to the reduced kernel-water content and/or altered enzymatic activity (Bai et al., 2017).

76 AI and PV must comply with the quality standards established for cold-pressed and non-  
77 refined macadamia oils which require a maximum PV value of  $3.5 \text{ meq}\cdot\text{kg}^{-1}$  and a maximum AI  
78 value of 0.5% (SAMAC, 2015). The standard methods for PV and AI analyses are slow and time-  
79 consuming, requiring precise quantification of the sample, dissolution in solvents, and titration with  
80 standardized solutions, and are also relatively costly when used for industrial process monitoring  
81 (Cozzolino, Murray, Chree & Scaife, 2005).

82 Near-infrared spectroscopy (NIRS) is widely employed for oxidation and moisture content  
83 determination in oilseeds and grains and has the potential to become a powerful tool in lipid  
84 oxidation analysis, especially with chemometric statistical evaluations. Non-destructive techniques  
85 have great potential for shelled- and unshelled- macadamia nut sorting, and NIRS is such an  
86 alternative, due to its long-term application to assess food-product quality parameters (Osborne,  
87 Fearn & Hindle, 1993).

88 Canneddu, Júnior & Teixeira (2016) used FT-NIR spectroscopy without any pre-processing  
89 to study the quality of shelled- and unshelled macadamia nuts, obtaining a coefficient of  
90 determination ( $R^2_p$ ) of 0.72 for PV prediction and an  $R^2_p$  of 0.80 for AI prediction. Guthrie, Greensill,  
91 Bowden & Walsh (2005), evaluated the use of NIRS to assess the quality of macadamia kernels and  
92 found an acceptable oil content prediction (root mean square error of cross validation  $\text{RMSE}_{\text{CV}} =$   
93 2.5% and  $R^2 > 0.98$ ), and moisture content prediction ( $\text{RMSE}_{\text{CV}} < 0.2\%$ , and  $R^2 > 0.97$ ).

94 As NIRS is a fast and non-destructive method used in industrial quality control processes, the  
95 objective of this study was to evaluate its feasibility as an analytical method to improve the quality  
96 control of macadamia-nuts in post-harvest operations.

97

## 98 **2. Materials and Methods**

### 99 **2.1. Plant Material**

100 Twelve kilograms (kg) of intact dehusked macadamia (*Macadamia integrifolia* Maiden &  
101 Betcher) nuts from each of the following cultivars, HAES 344 'Kau', HAES 660 'Keaau', IAC 4-12  
102 B, and IAC Campinas B, were obtained from a commercial orchard located in Jaboticabal, São  
103 Paulo, Brazil, (21° 10' 14" South, 48° 37' 45" West, 600 m altitude) during the 2017 harvest season  
104 (March, April, and May).

### 105 **2.2. Experimental set-up**

106 Immediately after the harvests (n=3), 4 kg of intact dehusked nuts were submitted to oven-  
107 drying (Innova 4230, Edison, USA) in four steps: *i.* control (without drying), *ii.* 4 days at 30 °C, *iii.* 2  
108 more days at 40 °C, and *iv.* 1 more day at 60 °C, to achieve the kernel moisture content of 1.5%  
109 stated by Mason (1995). The drying procedure was similar to what the macadamia industry uses in  
110 Brazil.

111 Initially (day 0) and at each drying step (4, 6, and 7 days), intact nuts (40 g) were withdrawn  
112 from the oven and the shell manually removed using a vice (N3, Schulz, Brazil). Unshelled nuts had  
113 their moisture content (%) determined following the A.O.A.C. (1997) standard method, Table 2.

114 Our experiment was set up as a completely randomized block design in a factorial  
115 arrangement 3 (harvests: March, April, and May) x 4 (cultivars: HAES 344 'Kau', HAES 660  
116 'Keaau', IAC 4-12 B, and IAC Campinas B) x 3 (drying steps: 0 – control, 4 days at 30 °C, 2 more  
117 days at 40 °C, and 1 more day at 60 °C) with 5 repetitions, totaling 240 unshelled nut samples.

### 2.3. NIR spectra acquisition

The NIR spectra were taken on day 0, 4, 6, and 7 of the drying steps, on the surface of ~ 40 grams of unshelled macadamia nuts from each cultivar. The nuts were placed into a measuring cup and two NIR spectra were obtained on the range 866 – 2,530 nm, at a resolution of 2 nm and 64 scans, after temperature stabilization at ~25 °C, using a Bruker NIR spectrometer (Model Tango, Ettlingen, Germany) in reflectance mode. The NIR spectra were averaged, and the mean spectra were processed, and the calibration models were performed using the MATLAB®, R2012b (MathWorks, USA) software.

### 2.4. Reference analyses: PV and AI

After drying, all samples were frozen at –20 °C, stored into plastic bags (~ 40 g of nuts per period and cultivar), to avoid oxidation. The oil present in the nuts was extracted daily to avoid supplementary oxidation, using the procedure reported by Canneddu, Júnior & Teixeira (2016). Briefly, the kernels were wrapped in nylon fabric and put into a stainless-steel vial with a tightly adjusted stainless-steel piston. The piston was pressed until free oil could be seen. The oil was rapidly transferred to a Falcon tube (BD Falcon™, BD Biosciences, Mass., USA) and used for the oxidation analysis.

*PV*: The peroxide value was determined using the official method published by the A.O.A.C. (1997). Briefly, 5 g of freshly pressed macadamia oil was poured into a 125 mL Erlenmeyer. Thirty mL of acetic acid-chloroform solution (3:2 v/v) was added and the flask was agitated until oil dissolution. Then, 0.5 mL of saturated potassium iodide solution was added, mixed, and let to rest for 1 minute. Thirty mL of distilled water was added, and the solution titrated with 0.01 N sodium thiosulfate. PV was expressed as active-oxygen milliequivalents per kilogram (meq·kg<sup>-1</sup>). The descriptive statistics of all samples is shown in Table 1.

*AI*: The acidity index was also determined using the A.O.A.C. (1997) official method. Five mL of freshly pressed macadamia oil was poured into a 125 mL Erlenmeyer and 25 mL of ether-

ethanol (2:1 v/v) solution was added. NaOH 0.01 M was used to neutralize the free fatty acids present in the oil with phenolphthalein (0.1%) as indicator. AI was expressed as the percentage of oleic acid (%). The descriptive statistics of all samples are shown in Table 1.

## 2.5. Data analysis

### 2.5.1. Chemometrics: multivariate analysis

All the NIR spectra were handled, and the calibration models developed, using the MATLAB®, R2012b (MathWorks, USA) software with the PLS Toolbox version 7.9.3 (Eigenvector Research, Inc., USA). Prior to the spectral pre-processing, all NIR spectra were analyzed to identify and eliminate outliers based on Hotelling's  $T^2$  and Q-residuals statistics (Bro and Smilde, 2014).

NIR datasets are often very large and highly complex and, consequently, need to be pre-processed. Therefore, prior to model development, the full spectra were pre-processed using multiplicative scatter correction (MSC), standard normal variate (SNV), Savitzky-Golay smoothing (SG), and derivatives. Then, the spectral datasets were correlated with the PV and AI values using Partial Least Squares (PLS) regression and full cross-validation. The NIR spectra were divided into two groups: the calibration set (n=154) and the validation set (n=66), using the classic Kennard–Stone selection algorithm (Kennard and Stone, 1969). In order to evaluate the performance of the calibration models, the root mean square error of cross-validation (RMSE<sub>CV</sub>) and root mean square error of prediction (RMSE<sub>P</sub>) were calculated, according to the following equation:

$$\text{RMSEC or RMSEP} = \sqrt{\frac{\sum_{i=1}^{np} (y_i - y_i')^2}{n - K - 1}}$$

Where:  $y_i$  is the value predicted by the multivariate model,  $y_i'$  the reference value, and  $n$  the number of samples.

The performance of the calibration models was also evaluated based on the determination coefficient  $R^2$ , both for the calibration and validation sets (Pasquini, 2003).

### 2.5.2. Univariate analysis



167 The data was subjected to analysis of variance (ANOVA) using the Agrostat (Barbosa and  
168 Maldonado Júnior, 2015) software and the means were compared using Tukey's test with 5%  
169 probability.

170

### 171 **3. Results and Discussion**

#### 172 **3.1. Nut quality during drying**

173 The moisture, dry matter contents (%), PV and AI of the unshelled macadamia nuts of the  
174 different cultivars, determined during drying, are shown in Table 2.

175 No significant moisture content differences were observed between nuts from different  
176 cultivars, but during the drying process moisture content became significantly lower (Table 2). The  
177 moisture content between cultivars ranged from 5.97% in HAES 344 to 7.24% in IAC Campinas B.  
178 During the drying process the highest moisture content was observed on day 0 (13.81%); after 4 days  
179 at 30 °C this value significantly ( $P < 0.05$ ) decreased to 6.09%. The moisture content decreased to  
180 4.13% after 2 days at 40 °C, and to 2.62% after 1 day at 60 °C, but without significant differences.  
181 The dry matter content showed a similar trend, but in the opposite direction, increasing during drying  
182 due to the dehydration process (Table 2).

183 According to Mason (2000) and Cavaletto (1983), macadamia nuts at harvest can have a  
184 moisture content as high as 30% and it is essential to reduce it to 1.5% to prevent hydrolytic activity  
185 and mold development. O'Hare, Hidden, Burton & Salmon (2004), reported that the nut-in-shell  
186 moisture content should be reduced to 8–10% within two weeks of harvest to prevent quality  
187 deterioration, mainly to control mold development, rancidity, off-flavors, and reduced shelf-life. In  
188 this regard, only the nuts from day 0 presented a moisture content above this range, and after only 4  
189 days at 30 °C the moisture content reached 6.09%, inside the 8–10% range.

190 The moisture content control during macadamia nut handling is extremely important as  
191 rancidity can occur rapidly (Cavaletto, 1981; Cavaletto, 1986; Wall, 2013). According to Kaijser,

192 Dutta & Savage (2000), macadamia oil stability ranged from 3.6 to 19.8 h using the Rancimat test.  
193 Macadamia nuts are susceptible to rancidification due to their high oil content. Cavaletto (1983)  
194 reported an oil content of 72% and Kaijser, Dutta & Savage (2000) observed a range from 69 to  
195 78%. In addition, the macadamia oil is highly monounsaturated (Ako, Okoda & Gray, 1995) and  
196 contains high levels of oleic (18:1), palmitoleic (16:1), and palmitic (16:0) acids (Kaijser, Dutta &  
197 Savage, 2000). Therefore, because drying was conducted immediately after harvest it resulted in a  
198 rapid moisture content reduction that improved the oxidative stability of the kernel, as evidenced by  
199 the low PV and AI values (Table 2).

200 No significant PV differences were observed neither from different cultivars nor during the  
201 drying process (Table 2). PV ranged from 1.08 meq·kg<sup>-1</sup> in the nuts from the IAC 4-12 cultivar to  
202 0.73 meq·kg<sup>-1</sup> in those from HAES 344 (Table 2). During drying the PV ranged from 1.27 in day 0  
203 to 0.66 meq·kg<sup>-1</sup> after 4 days at 30 °C, 2 days at 40 °C, and 1 day at 60 °C (Table 2). The observed  
204 PVs were very low compared to those of the macadamia quality standards. According to Mason,  
205 Nottingham, Reid & Gathambiri (2004) the AMS Guidelines indicate a maximum PV of 5.0  
206 meq·kg<sup>-1</sup> for oil from raw and roasted macadamia kernels. According to SAMAC (2015), raw  
207 macadamia nuts must have PV less than 3.5 meq·kg<sup>-1</sup>. Macadamia oil is considered moderately to  
208 completely rancid when its PV reaches 6.0 meq·kg<sup>-1</sup> (McConachie, 1996).

209 PV can be influenced by the cultivar as each cultivar has a different nut polyunsaturated fatty  
210 acid composition (Kaijser, Dutta & Savage, 2000). Processing also influences PV. Canneddu, Júnior  
211 & Teixeira (2016), reported lower PVs (5.6–5.5 meq·kg<sup>-1</sup>) in freshly harvested intact and ground  
212 macadamia nuts, when compared to half kernels from late harvest (10.7–18.8 meq·kg<sup>-1</sup>). Oxidative  
213 rancidity is very important in lipid stability and since macadamia nuts have a low concentration of  
214 polyunsaturated fatty acids (3.0–4.7%) peroxides production is generally low in unstored kernels  
215 (Kaijser, Dutta & Savage, 2000). However, it can be a quality problem during drying

216 (Borompichaichartkul, Luengsode, Chinprahast & Devahastin, 2009) and long-term storage  
217 (Sinanoglou et al., 2014).

218       Regarding AI, no significant ( $P > 0.05$ ) differences between different cultivars were observed  
219 and during drying process the AI decreases significantly (Table 2) ranging from 0.75% in nuts from  
220 the IAC Campinas B cultivar to 0.66% in those from HAES 344. During the drying process the  
221 highest AI was observed at day 0 (1.0 %); after 4 days at 30 °C this value significantly ( $P < 0.05$ )  
222 decreased to 0.63%. AI decreased to 0.61% after 2 days at 40 °C and to 0.56% after 1 day at 60 °C,  
223 but without significant differences (Table 2). This reduction of AI during the drying process could be  
224 related to the inactivation of the enzymes involved in the hydrolytic reactions (Silva et al., 2011),  
225 which are directly related to the moisture content present in the kernel (Silva, Maximo, Marsaioli  
226 Júnior & Silva, 2007). Although the drying process was conducted immediately after harvesting, AI  
227 values were above the maximum levels recommended for macadamia oil.

228       According to SAMAC (2015), the maximum AI should be 0.5%. McConachie (1996)  
229 considered that high quality macadamia oil should have AI of only 0.1–0.3%, but Arnett (1995)  
230 considered an AI of 0.9% as acceptable. Therefore, hydrolytic rancidity seems to be a more  
231 important factor for macadamia nuts quality, as its development of rancid flavors and odors can take  
232 place rapidly even during prompt drying.

233

### 234 **3.2. NIR spectra description**

235       A preliminary analysis of the raw NIR spectra showed very similar trends for the macadamia  
236 nuts from all cultivars. Carvalho et al. (2018) also observed similarities of different macadamia nut  
237 NIR spectra and the classification of shelled nuts was only possible using sophisticated chemometric  
238 techniques. Because the raw NIR spectra presented broad light scattering they were pre-processed  
239 with MSC and SNV plus Savitsky-Golay smoothing to allow the identification of four main peaks at  
240 1200, 1500, 1800, and 2000 nm. The absorption bands around 1200 nm arise from the second

241 overtones of CH stretching vibrations, while those at 1700–1800 nm are attributable to the first  
242 overtones of CH stretching vibrations of  $-\text{CH}_3$ ,  $-\text{CH}_2-$  and  $-\text{HC}=\text{CH}$  (Armenta and La Guardia,  
243 2007). The wavelength region situated at 2200–2500 nm is mainly related to the oxidation and  
244 hydrolytic degradation of lipids (Cozzolino, Murray, Chree & Scaife, 2005).

245 During the drying process the NIR spectra changed mainly in the regions where the water  
246 bands are present (1400–1440 nm and 1900–1950 nm) (Sundaram, Kandala, Holser, Butts &  
247 Windham, 2010), indicating that there was a reduction in moisture content, as can be seen in Table 2.

248 Principal component analysis (PCA) was used to observe the spectral differences between  
249 drying processes and macadamia nut cultivars, helping to observe possible groupings between the  
250 samples due to their similarities (Cozzolino, Cynkar, Shah & Smith, 2011). The scores of the two  
251 main principal components (PCs) obtained with the NIR spectra without preprocessing can be  
252 observed in Figure 1. Regarding the drying process, PC2 grouped the macadamia nut NIR spectra  
253 from day 0 and 7 (Figure 1A), as there was a significant moisture content difference ( $P < 0.05$ )  
254 between day 0 and day 7 (Table 2). It was not possible to observe any grouping among macadamia  
255 nut cultivars (Figure 1B), with PC1 and PC2 representing 97% of the data explained variance. This  
256 result indicates that there was similarity between macadamia nut cultivars, which may be related to  
257 climatic factors and/or for sharing the same pollination since they originated from the same orchard  
258 (Carvalho et al. 2018).

259

### 260 3.3. Oxidative stability: PV and AI

261 The PV of the samples ranged from 0.14 to 5.41  $\text{meq}\cdot\text{kg}^{-1}$  and the AI from 0.18 to 2.26%  
262 (Table 1) in the calibration set. As shown in Table 3, the best PLS model for PV ( $\text{RMSE}_C = 0.56$   
263  $\text{meq}\cdot\text{kg}^{-1}$ ,  $R_c^2 = 0.57$ ,  $\text{SEP} = 0.55 \text{ meq}\cdot\text{kg}^{-1}$ ,  $\text{RMSE}_P = 0.60 \text{ meq}\cdot\text{kg}^{-1}$ ,  $R_P^2 = 0.002$ ) was obtained  
264 when the NIR spectra were pre-processed with SG second derivative. The predicted vs. measured PV  
265 values can also be seen in Figure 2. Canneddu, Júnior & Teixeira (2016) studying the quality of

266 unshelled macadamia nuts by NIRS found higher SEP values ( $3.45 \text{ meq}\cdot\text{kg}^{-1}$ ), but with a greater  
267 variation in the PV values ( $4.6\text{--}26.8 \text{ meq}\cdot\text{kg}^{-1}$ ) of the calibration set. Better PV prediction by NIRS  
268 was reported by Armenta and La Guardia (2007) in edible oils of different types and origins, with  
269 RMSEP values of  $1.87 \text{ meq}\cdot\text{kg}^{-1}$  and  $R^2_c$  of 0.99. However, intact macadamia nuts are a more  
270 heterogeneous matrix as compared to vegetable oils and this could have affected the results,  
271 especially the lower determination coefficients (Table 3).

272 In contrast, considering the maximum PV quality limit of  $3.0 \text{ meq}\cdot\text{kg}^{-1}$  (SAMAC, 2015), the  
273 SEP value ( $0.55 \text{ meq}\cdot\text{kg}^{-1}$ ) represented 18% of this recommendation. Therefore, the PV prediction  
274 method can be considered excellent as its total error was of magnitude 25% or less (McFarren,  
275 Lishka & Parker, 1970).

276 Regarding AI, the best PLS model was obtained when the NIR spectra were pre-processed  
277 using SG second derivative ( $\text{RMSE}_c = 0.25\%$ ;  $R_c^2 = 0.56$ ;  $\text{SEP} = 0.29\%$ ;  $\text{RMSE}_p = 0.30\%$ ;  $R_p^2 = 0.018$   
278 with 7 latent variables), Table 3 and Figure 3. As indicated in Table 3, the SEP and RMSEP  
279 differences were not significant which could be taken as an indication that the prediction precision of  
280 the PLS regression was high (Amodio, Ceglia, Chaudhrya, Piazzolla & Colellia, 2017), although  $R^2$   
281 was low. Similar RMSEP values (0.35%) were reported by Mba, Adewale, Dumont & Ngadi (2014)  
282 in palm and canola oils with NIR spectra pre-processed using SG first derivative, but their  $R^2$  was  
283 much greater (0.99). Better results were reported by Armenta and La Guardia (2007) in olive oil  
284 ( $\text{RMSEP} = 0.083\%$  and  $R^2 = 0.99$ ); once again, the difference between food matrixes must be taken  
285 into consideration. Although the  $R^2$  values were below 0.70, considering the AI level of 0.5% as the  
286 maximum quality limit of macadamia nuts (SAMAC, 2015), the SEP value represented 28% of this  
287 limit. Consequently, the AI prediction as an analytical method can be considered acceptable as its  
288 total error was of the magnitude of 50 % or less (McFarren, Lishka & Parker, 1970).

289 The moisture prediction results (Table 3, Figure 4) indicate that the best calibration models  
290 for moisture content of unshelled nuts were obtained using SG + 2nd derivative ( $R_c^2 = 0.66$ ;  $\text{RMSE}_c$

291 = 2.33%). Better results were reported by Guthrie, Greensill, Bowden & Walsh (2005) who found  
292 moisture content prediction models with RMSEcv of 0.11% and  $R^2$  of 0.79. Their results were  
293 improved by using ground nuts (RMSEcv = 0.06%  $R^2$  = 0.95), and these differences could be due to  
294 the lack of moisture homogeneity within our intact kernels; grinding should have reduced this  
295 variation. Sumdaram, Kandala, Holser, Butts & Windham. (2010) also reported better results for in  
296 shell peanuts using NIRS (RMSEp = 1.38% and  $R^2$  = 0.98), but the moisture content range was  
297 much higher (6.18 - 21.69 %) and should be taken into consideration.

298

#### 299 **4. Conclusion**

300 Cultivars had little effect on the oxidative stability of intact unshelled macadamia nuts based  
301 on PV and AI. On the other hand, the drying process affected mostly the AI as the PV did not present  
302 any modifications during drying.

303 The NIR spectra were similar between cultivars, but the absorption bands changed during the  
304 drying process and the characteristic oil bands could be seen when the MSC, SNV, and SG  
305 smoothing derivatives were applied.

306 The PLS calibration models for PV and AI were considered useful since the error is lower of  
307 the quality limits. Therefore, NIRS can be used to assess the oxidative stability of intact macadamia  
308 nuts, but further investigation must be done to address other sources of variability to improve the  
309 robustness of the prediction models.

310

#### 311 **Acknowledgment**

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314

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422

423 \*These references were fundamental for the discussion of the article, since they deal with  
424 works similar to the above, using macadamia nuts, near infrared spectroscopy combined with  
425 chemometrics and oxidative evaluation of nuts, clearly exposing the quality parameters of  
426 macadamia nuts.

427

## 428 **Tables**

429

430 **Table 1.** Descriptive statistics of unshelled macadamia nuts of the calibration and validation sets  
 431 obtained using the classic Kernnard-Stone selection algorithm.

Description	Total	Mean	Maximum	Minimum	SD <sup>a</sup>
Calibration set	154*				
Peroxide value (meq.kg <sup>-1</sup> )	154	0.90	5.41	0.12	0.82
Acidity index (%)	154	0.68	2.26	0.20	0.35
Validation set	66*				
Peroxide value (meq.kg <sup>-1</sup> )	66	0.86	2.49	0.14	0.58
Acidity index (%)	66	0.60	1.65	0.18	0.30

432 <sup>a</sup>SD = standard deviation. \*from the total of 240 samples, 20 were lost during oil extraction which  
 433 corresponds a total of 220 samples.

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438 **Table 2.** Moisture, dry matter, peroxide value, and acidity index of unshelled macadamia  
 439 (*Macadamia integrifolia* Maiden & Betche) from different cultivars during drying process.

	Moisture (%)	Dry matter (%)	Peroxide value (meq.kg <sup>-1</sup> )	Acidity index (%)
<b>Harvests (B)</b>				
March	7.2±3.7	92.6±3.6	1.15±1.05	0.69±0.40
Aphril	3.7±5.3	94.4±3.7	0.84±0.54	0.66±0.28
May	2.6±1.5	97.4±1.5	0.66±0.35	0.61±0.32
<b>Cultivars (C)</b>				
IAC 4 12	7.1±7.9	92.8±7.9	1.08±0.67	0.72±0.37
IAC Campinas B	7.2±4.4	92.5±4.6	0.96±0.77	0.75±0.36
HAES 660	6.4±5.5	93.9±5.4	0.90±0.54	0.67±0.22
HAES 344	6.0±5.1	93.5±5.1	0.73±0.37	0.66±0.20
<b>Drying period (D)</b>				
0	13.8±6.9 <sup>a</sup>	86.2±6.4 <sup>c</sup>	1.27±0.97	1.01±0.43 <sup>a</sup>
4	6.1±2.7 <sup>b</sup>	93.3±3.1 <sup>b</sup>	0.93±0.61	0.63±0.27 <sup>b</sup>
6	4.1±1.5 <sup>b</sup>	95.9±1.5 <sup>ab</sup>	0.81±0.36	0.61±0.12 <sup>b</sup>
7	2.6±1.2 <sup>b</sup>	97.4±1.2 <sup>a</sup>	0.66±0.27	0.56±0.21 <sup>b</sup>
<b>Interaction</b>				
C x D	NS	NS	NS	NS

440 Average values with the same letter in the columns are not statistically different by Tukey's test

441 (p<0,05). Values in the column without letter are not statistically different by Tukey's test (P<0,05).

442 NS, non-significant interaction.

443

444

446 **Table 3.** Statistical parameters for the optimal prediction models for peroxide value, acidity index,  
 447 and moisture of unshelled macadamia (*Macadamia integrifolia* Maiden & Betche) from different  
 448 cultivars during drying process.

<b>Peroxide value (PV)</b>		<b>mequiv. O<sub>2</sub> kg<sup>-1</sup> units</b>				
<i>Pre- Processing</i>	Calibration		Validation			
	RMSE <sub>c</sub>	R <sub>c</sub> <sup>2</sup>	RMSE <sub>p</sub>	SEP	Bias	RE(%)
Raw	0.78	0.18	0.47	0.42	0.21	66.57
SG+SNV	0.76	0.21	0.50	0.48	0.18	72.16
SG+MSC+baseline	0.78	0.17	0.51	0.45	0.23	72.33
SG+2 <sup>nd</sup> derivative	0.56	0.57	0.60	0.55	0.26	86.04
<b>Acidity Index (AI)</b>		<b>% (w/w) units</b>				
<i>Pre- Processing</i>	Calibration		Validation			
	RMSE <sub>c</sub>	R <sub>c</sub> <sup>2</sup>	RMSE <sub>p</sub>	SEP	Bias	RE(%)
Raw	0.36	0.06	0.22	0.16	0.15	38.89
SG+SNV	0.36	0.08	0.23	0.16	0.16	40.93
SG+MSC+baseline	0.35	0.12	0.25	0.19	0.16	44.80
SG+2 <sup>nd</sup> derivative	0.25	0.56	0.30	0.29	0.10	54.93
<b>Moisture</b>		<b>% (w/w) units</b>				
<i>Pre- Processing</i>	Calibration		Validation			
	RMSE <sub>c</sub>	R <sub>c</sub> <sup>2</sup>	RMSE <sub>p</sub>	SEP	Bias	RE(%)
Raw	3.21	0.23	3.01	3.03	0.12	49.65
SG+SNV	3.22	0.23	3.12	3.15	-0.01	51.51
SG+MSC+baseline	3.26	0.21	3.20	3.23	-0.15	52.83
SG+2 <sup>nd</sup> derivative	2.13	0.66	3.23	3.26	-0.07	53.31
<b>Dry Matter</b>		<b>% (w/w) units</b>				
<i>Pre- Processing</i>	Calibration		Validation			
	RMSE <sub>c</sub>	R <sub>c</sub> <sup>2</sup>	RMSE <sub>p</sub>	SEP	Bias	RE(%)
Raw	3.30	0.26	3.07	3.09	-0.15	3.24
SG+SNV	3.30	0.25	3.17	3.20	0.01	3.34
SG+MSC+baseline	3.34	0.24	3.26	3.28	0.15	3.43
SG+2 <sup>nd</sup> derivative	2.19	0.67	3.31	3.33	0.01	3.48

449 mequiv = milliequivalent; SG = Savitzky-Golay smoothing; SNV = standard normal variate; MSC =  
 450 multiplicative scatter correction; RMSEC = root mean square error of calibration; RMSEP = root  
 451 mean square error of prediction; R<sub>c</sub><sup>2</sup> = coefficient of determination for calibration; SEP = standard  
 452 error for prediction; RE = relative error.

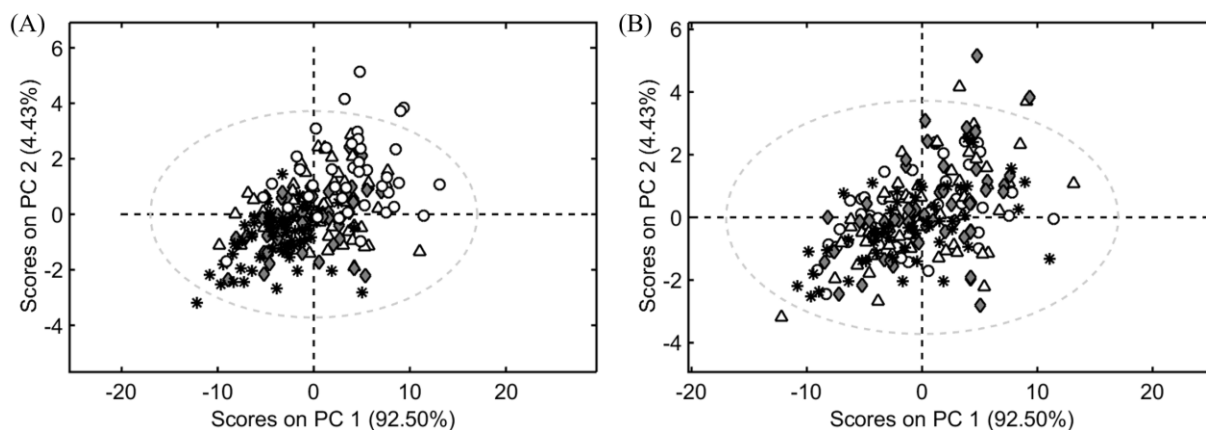
454

# 455 **Figures**

456

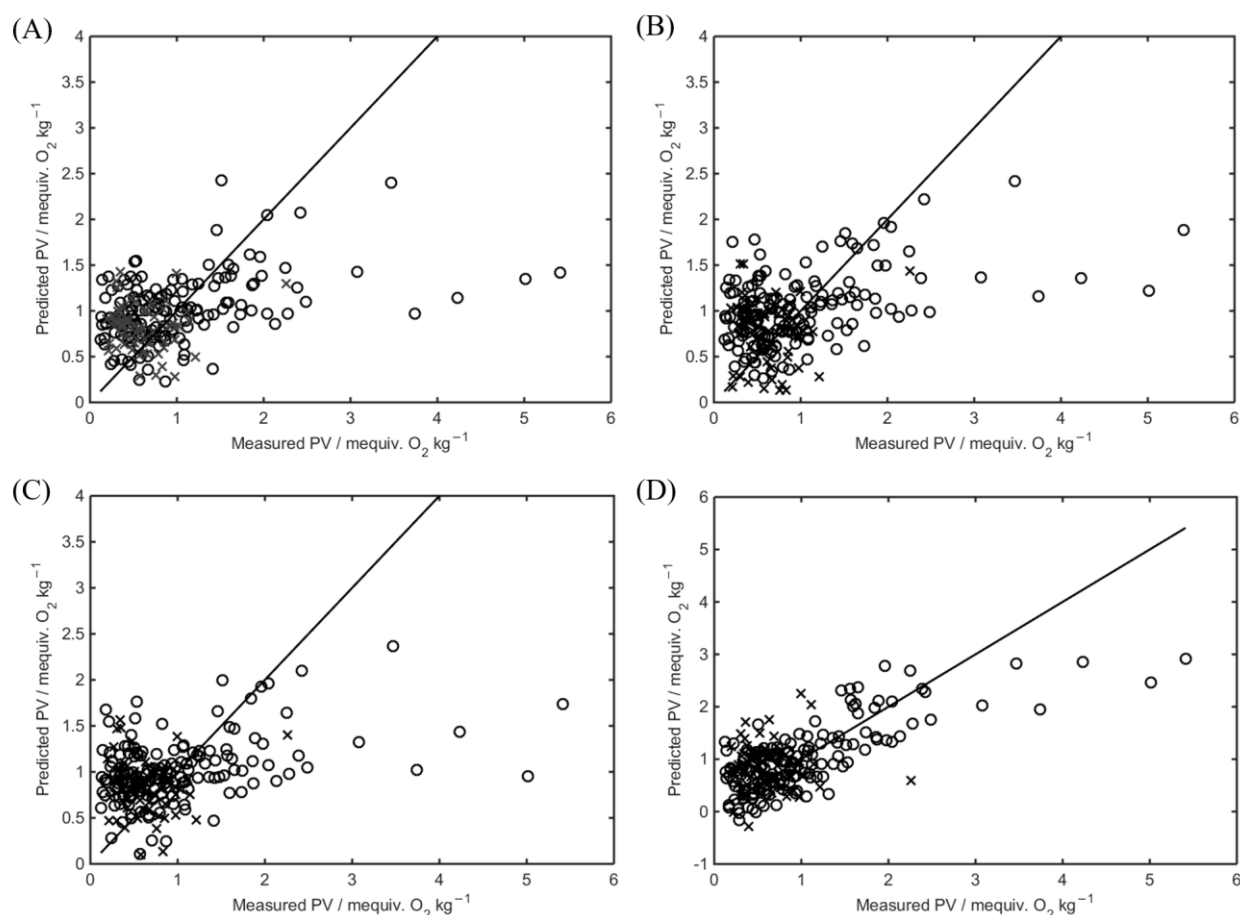
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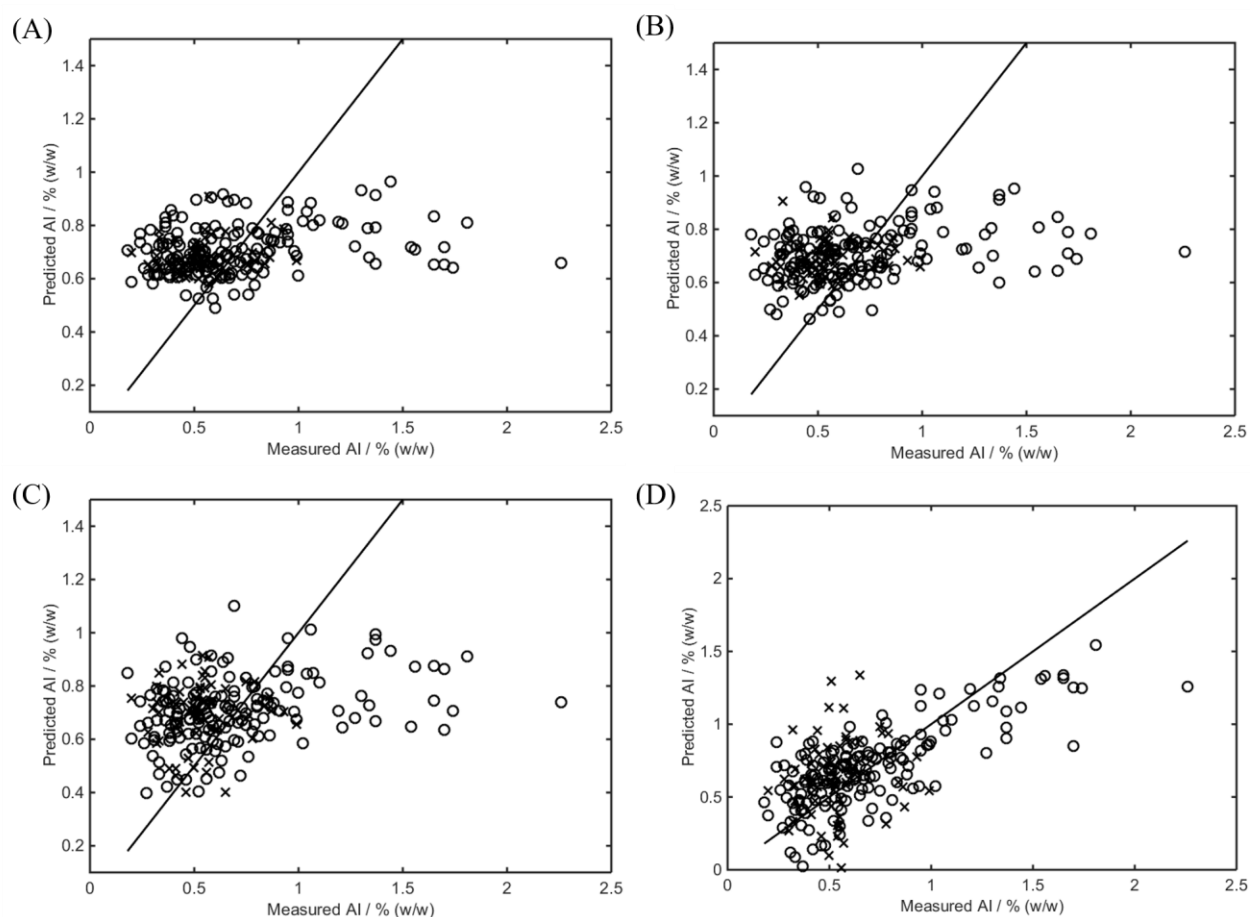
460 **Figure 1.** (A) scores of the PCA for the NIR spectra of drying process (Day 0 [○], 4 [▲], 6 [◇], 7  
 461 [\*]); (B) scores of the PCA for the NIR spectra of unshelled macadamia (*Macadamia integrifolia*  
 462 Maiden & Bette) nuts from different cultivars (IAC 4-12 [◇], IAC Campinas B [\*], HAES 660 [▲],  
 463 HAES 340 [○]).



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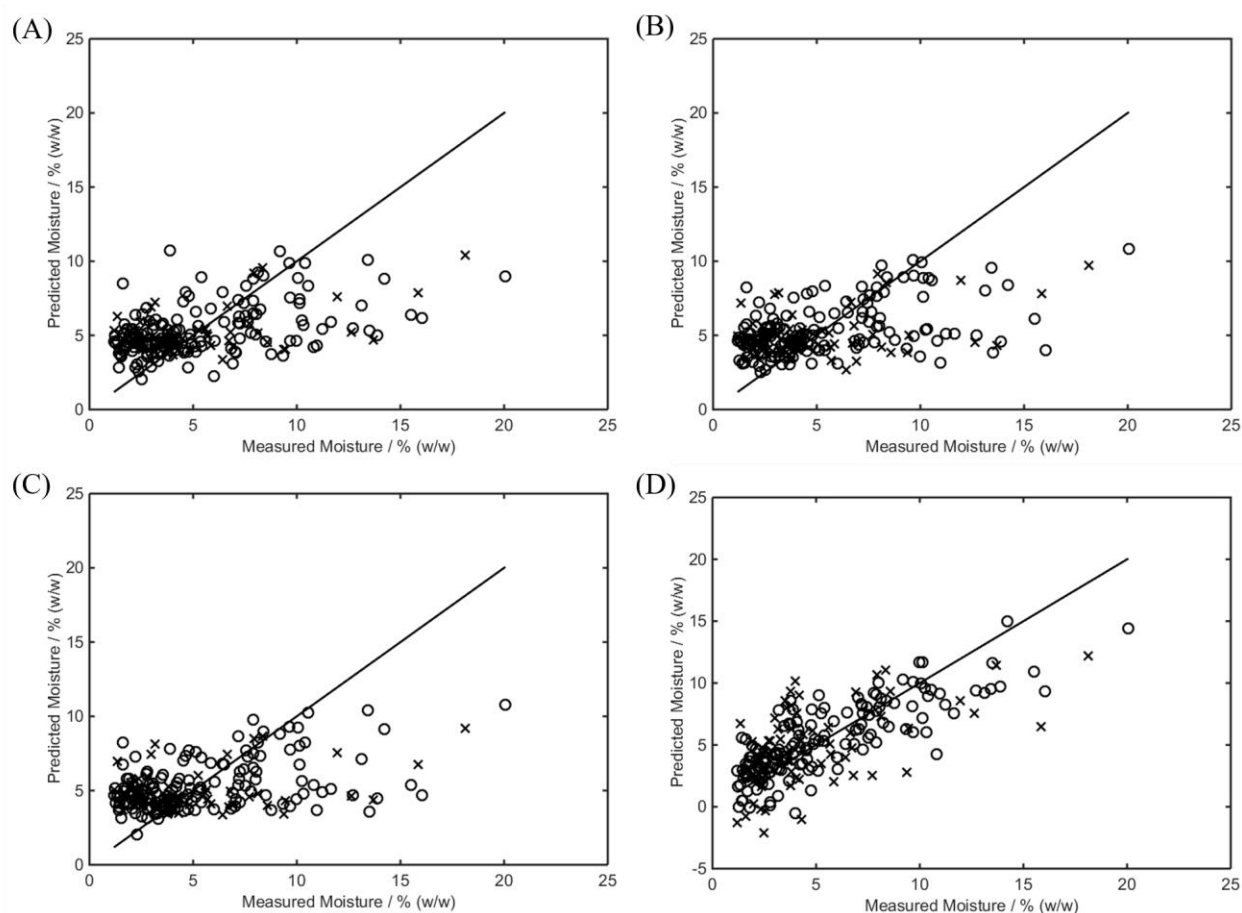
465 **Figure 2.** Measured *versus* predicted peroxide value (PV) for the calibration (o) and validation (x)  
 466 sets using PLS regression with the following pre-processing: (A) raw spectra; (B) SG+SNV; (C)  
 467 SG+MSC+baseline; (D) SG+2<sup>nd</sup> derivative. SG = Savitzky-Golay smoothing; SNV = standard  
 468 normal variate; MSC = multiplicative scatter correction.





469

470 **Figure 3.** Measured *versus* predicted acidity index (AI) for the calibration (o) and validation (x) sets  
 471 using PLS regression with the following pre-processing: (A) raw spectra; (B) SG+SNV; (C)  
 472 SG+MSC+baseline; (D) SG+2<sup>nd</sup> derivative. SG = Savitzky-Golay smoothing; SNV = standard  
 473 normal variate; MSC = multiplicative scatter correction



474

475 **Figure 4.** Measured *versus* predicted moisture for the calibration (o) and validation (x) sets using  
 476 PLS regression with the following pre-processing: (A) raw spectra; (B) SG+SNV; (C)  
 477 SG+MSC+baseline; (D) SG+2<sup>nd</sup> derivative. SG = Savitzky-Golay smoothing; SNV = standard  
 478 normal variate; MSC = multiplicative scatter correction.